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The Use of a Biological Adhesive To Achieve Sutureless Epikeratophakia

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Summary

An adhesive made of concentrated human fibrinogen produced from samples of single donor blood by a simple cryoprecipitation method was tested for its ability to bond lamellar corneal buttons *in vitro* and found to have a bond strength of 140 gm/cm^2. The adhesive was successfully used to attach experimental epikeratophakia grafts in a rabbit model. The results were similar to those reported from an earlier trial using a commercially available adhesive prepared from pooled multi-donor human plasma. This experimental trial of single-donor adhesive demonstrates the possibility of successful use of autologous fibrinogen adhesive in human epikeratophakia. Autologous adhesive would remove the possible threat of transmitted disease posed by multi-donor adhesive and avoid immune reactions to foreign proteins.

Experimental Aims

In epikeratophakia, an optically preshaped lenticule made from human donor cornea is permanently fixed to the recipient cornea by sutures. Suturing is time consuming and if sutures are incorrectly placed or tensioned, may give rise to astigmatic errors or alter the refractive result impairing the visual outcome.[1] Sutures may cause irritation and once healing has been established they are usually removed, which may necessitate a second anaesthetic in children. The object of this study was to determine whether single-donor fibrinogen adhesive could be used to achieve sutureless experimental epikeratophakia in a rabbit model.

Tissue Adhesives

Synthetic Adhesives

Cyanoacrylate glue has proved its usefulness in the repair of corneal perforations,[2,3,4] but it has been clearly shown to be of little use in lamellar,[5] or penetrating keratoplasty.[6] In 1968 Cardaralli and Basu[5] performed a series of 49 experimental lamellar keratoplasties using cyanoacrylate glue and achieved graft retention in only ten. All grafts developed significant opacities, and where the glue had spread into the graft-host interface the graft became opaque and sloughed off within 48 hours.

Biological Adhesives

In 1944 Brown and Nantz[7] used the adhesive power of the fibrinogen found in high concentration in rabbit aqueous to seal corneal wounds in rabbits. In 1945 Katzin[8] reported graft retention in 86% of autologous rabbit penetrating keratoplasties secured only with aqueous fibrinogen with added topical thrombin; however it was considered that the risks of wound dehiscence and iris prolapse were too great for the technique to be applied to human keratoplasty. The promis-

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ing results reported[9,10] from a wide range of ophthalmic surgical procedures performed with fibrin coagulum were not followed up by clinical application.

Rosenthal *et al* introduced the concept of a platelet-fibrinogen-thrombin mixture and in rabbit experiments achieved a 75% retention rate of 4 mm diam. autologous, penetrating keratoplasty grafts[11] and a 50% retention rate in autologous, lamellar keratoplasty.[12] These results were no better than those of Katzin.[8]

Mechanisms of Action of Fibrinogen Adhesive

In the formation of a fibrin clot, thrombin causes fibrinogen to break down into fibrin monomers which then cross-link in the presence of Factor XIII and calcium ions to become stable fibrin polymer (Diagram 1).

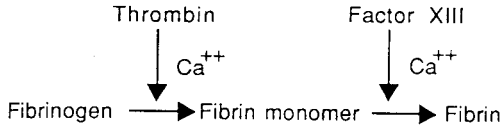

Diagram 1. *Pathway of fibrin clot formation.*

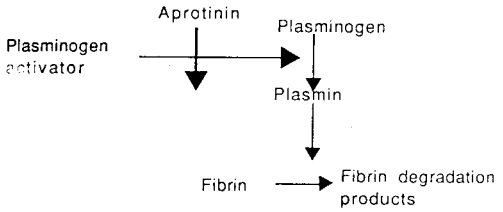

Diagram 2. *Pathway of fibrin degradation.*

Fibrinolysis in wound healing results from the action of plasmin on fibrin polymer. Plasmin in turn is a product of plasminogen found in normal plasma (Diagram 2). The adhesive has been shown to be completely absorbed once healing has taken place,[13] making it suitable for use in refractive surgery. The natural fibrinolytic action of plasmin can be blocked by aprotinin as a result of its inhibition of plasminogen activator. The addition of bovine aprotinin to commercial fibrinogen adhesive is intended to inhibit the lysis of the fibrin bond and prolong its duration of action.

Highly Concentrated Fibrinogen

Commercially prepared highly concentrated fibrinogen has been available in Europe for several years ('Beriplast'; Behringwerke AG, Marburg, W Germany and 'Tisseel' & 'Tissucol', Immuno AG, Vienna, Austria) and has been successfully applied to a wide variety of surgical techniques[14,15] including intraocular surgery.[16,17] In lamellar keratoplasty, Klemen *et al*[18] reported satisfactory graft retention, but because of separation of the marginal wound due to graft shrinkage, concluded that concentrated fibrinogen alone was insufficient to secure the graft without the addition of sutures. However we have recently reported its successful use in experimental epikeratophakia.[19] We found that a 70% graft retention could be achieved with 'Tisseel' when used in a rabbit model of epikeratophakia with slight modifications in the lenticule design and operative technique.

Transmission of Viral Infection

Commercially produced multiple-donor blood products are not licenced for clinical use in this country or in the USA[20] because of the risk of disease transmission.[21] This risk is reduced by selection of donors and screening of donor blood for hepatitis B surface antigen, HIV antigen and raised alanine aminotransferase. Lyophylised Tisseel is subjected to a special heat treatment at 60°C for 30 hours which has been shown to be effective at reducing the risk of transmission of viral disease[22,23,24] and should give a high margin of safety.[25,26] Although heat treatment of lyophilised blood products reduces the titre of viral infection,[26] it is not possible to be certain that all risk has been removed as tests for assaying viral titres have a finite limit to their sensitivity.[28]

An autologous adhesive, prepared from the patient's own blood, presents no threat of transmitted disease nor is it likely to provoke any immune reaction. It therefore presents an attractive solution to the problems presented by commercially prepared adhesive.

Preparation of Fibrinogen Concentrate

There are several methods of preparing concentrated fibrinogen. It can be precipitated out of plasma by the addition of various substances[21,29,30] but these methods were avoided because of their complexity and the

number of steps required, and because there may be a danger of creating opacities at the graft-host interface by the introduction of additional chemicals other than sodium citrate anti-coagulant.

Less than 50% of plasma can be recovered by our method of cryoprecipitation[33] and the final product from a 45 ml blood specimen is slightly less than 1 ml. Although our technique of epikeratophakia requires only 0.02 ml of fibrinogen adhesive, we found that it was not practical to handle volumes of fibrinogen concentrate of much less than 1 ml. Because it was difficult to obtain the relatively large volumes of blood from a rabbit, we did not attempt to prepare autologous rabbit adhesive. The ultimate object of the study was to assess the feasibility of the use of autologous fibrinogen glue in human epikeratophakia, consequently we prepared the adhesive from human, single-donor blood.

Materials and Methods

Adhesive Preparation

The single donor fibrinogen was obtained in the following manner: 45 ml of human venous blood was collected into a syringe containing 5 ml of 3.8% w/v sodium citrate, transferred into a single sterile centrifuge tube and spun at 2000 rpm for 10 minutes. The supernatant plasma was then carefully transferred to a second sterile centrifuge tube and frozen at −70°C. (1 hour). The frozen plasma was allowed to thaw overnight at a constant temperature of 4°C. The thawed plasma was spun in a cold centrifuge (4°C) at 3000 rpm for 15 minutes and the supernatant fluid was discarded, leaving a small white pellet of fibrinogen–rich precipitate in the apex of the centrifuge tube. On warming to room temperature, the precipitate liquified and a volume of 0.4–0.5 ml of concentrated fibrinogen was drawn up into a sterile 1 ml syringe and used immediately or refrozen to −70°C for later use.

In Vitro Adhesive Study

Fresh rabbit corneas were split at the mid stromal level and a trephine was used to punch 6 mm lamella buttons. Cyanoacrylate adhesive was used to fix the external surface of the lamella buttons onto perspex bases so that the dissected face of the buttons were exposed and kept free of cyanoacrylate. Thawed human cryoprecipitate was applied to the dried lamellar surfaces of a pair of buttons which were then carefully apposed together. A solution of bovine thrombin 500 IU/litre in a solution of calcium chloride 40mM/litre was applied and the two buttons were lightly pressed together for 2–3 minutes. After 10 minutes allowing the bond to gain strength, the two buttons were pulled directly apart with a steadily increasing force until separation occurred. The force required to separate the buttons was recorded on 20 different specimens.

Experimental Epikeratophakia Study

Lenticule Preparation

Donor tissue was lathed at room temperature[34] to produce lenticules with an overall diameter of 9 mm, an optic zone of 6 mm diameter and a back radius to correct high hypermetropia. Lenticules were lyophilised and stored in sterile vacuum vials until use.

Surgical Series

Adult male Dutch rabbits (2kg) underwent epikeratophakia surgery to the left eye only. The nictitating membrane was removed under anaesthetic, from the left eye and at least one week elapsed before epikeratophakia surgery was performed as a second procedure under general anaesthetic.

Anaesthesia

Surgery was performed using a premedication of intravenous midazolam (Hypnovel) 0.5ml/kg. Anaesthesia was maintained with fluothane 4% and oxygen 2 litre/min by mask. Post operative buprenorphine (Temgesic) 0.03mg/kg.was given subcutaneously following epikeratophakia surgery.

Surgical Technique

After fixation of the globe with traction sutures to two rectus muscles, cocaine 4% drops were applied to the cornea. A 6 mm trephine was used to mark the graft site, cutting only the most superficial corneal stroma. The epithelium was stripped only from within

the area marked by the trephine. The trephine mark was then deepened with a diamond blade to a depth of 0.1 to 0.2 mm. An angled lamellar dissector was used to split the cornea peripherally in a single plane extending through 360° for at least 1.5 mm from the corneal incision. The fibrinogen concentrate was applied directly to the dried de-epithelialised cornea and spread into the lamellar split. The washed lenticule was placed onto the adhesive on the host cornea and the wing-zone of the lenticule tucked into the lamellar split using an angled iris repositor. The positioning of the lenticule was corrected and any entrapped air or excess adhesive was removed by gently stroking towards the periphery of the lenticule. Any excess adhesive was removed from the surface of the eye and 4-5 drops of bovine thrombin 500 IU/mil in a solution of calcium chloloride 40mM/L were applied topically. A subconjunctival injection of methyl prednisolone (Depo-Medrone) 0.01 ml was given and topical chloramphenicol ointment applied. The lids were closed by a wide central, temporary tarsorrhaphy using two prolene 5/O marginal lid sutures with rubber bolsters. Post operative analgesia was administered.

Postoperative Treatment

Animals were examined daily and the tarsorrhaphy sutures were removed after an interval of 4 to 9 days. Subsequently the grafts were examined with a Topcon bio-microscope and anterior segment photography carried out at intervals. The animals were followed postoperatively for intervals ranging from 0 to 50 days. They were then killed by overdose of intravenous sodium pentobarbital. The operated eye was then immediately removed and placed in formalin fixative. The specimens were sectioned, stained with haematoxolyn and eosin and then examined by light microscopy.

Results

In Vitro Study

The *in vitro* adhesive study revealed a mean bond strength of 139.4 gm/cm^2, standard deviation 61.5gm/cm^2 at ten minutes.

Experimental Epikeratophakia Study

Animal No 5 died following an uneventful epikeratophakia operation, shortly after making an apparently normal recovery from the anaesthetic, for reasons which could not be ascertained by autopsy. The eye was removed for histological examination.

Of the eight surviving animals six grafts were successfully retained. (Table I). In both eyes that lost their graft, the anterior lamella of the host cornea overlying the graft wing zone was accidentally torn during surgery, resulting in a less stable graft. In all eyes, including those where the graft was lost, full re-epithelialisation of the graft or host cornea was established by the time the tarsorrhaphy was opened.

Some retained grafts suffered secondary epithelial breakdown with a varying amount of graft stromal melt. There were no significant interface opacities resulting from the adhesive.

Histopathology

Figure 1. shows a medium power view of a glued graft at 30 minutes postoperatively. The graft has no epithelium, as this was removed at an early stage in the preparation of the lenticule. The graft stroma appears to have a full keratocyte population. However these keratocytes have been killed during lyophilisation and the cellular debris has yet to be cleared by the host's phagocytes. At the graft-host interface, the fibrin adhesive can just be seen as a line of amorphous eosinophilic substance which is not promi-

Table I *Results of Single-Donor Fibrinogen Adhesive Trial*

Animal No.	*Tarsorrhaphy Opened (Days)*	*Follow-up (Days)*	*Graft Retained?*
1	9	17	yes
2	9	50	yes
3	4	33	yes
4	8	33	yes
5	0	0	yes
6	6	26	yes
7	6	26	no
8	9	22	yes
9	9	22	no

Fig. 1. *Shows the central graft stroma and glued graft-host interface at 30 minutes post-operatively. Haematoxylin-Eosin; original magnification, × 25.*

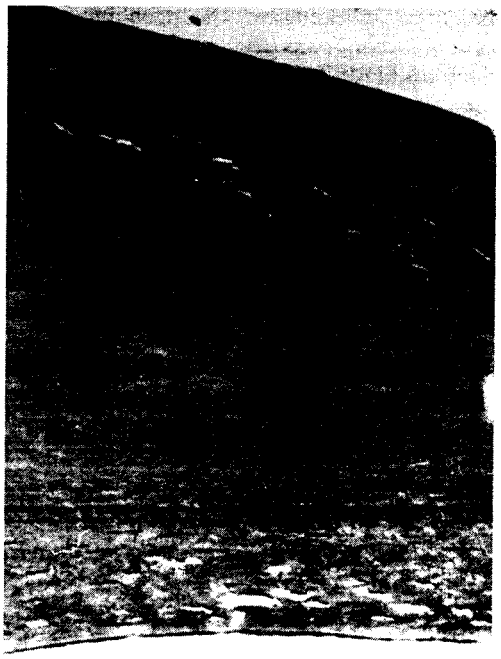

Fig. 2. *Shows the wing–zone of a glued epikeratophakia graft at 30 minutes post-operatively. Heamatoxylin–Eosin; original magnification, × 10.*

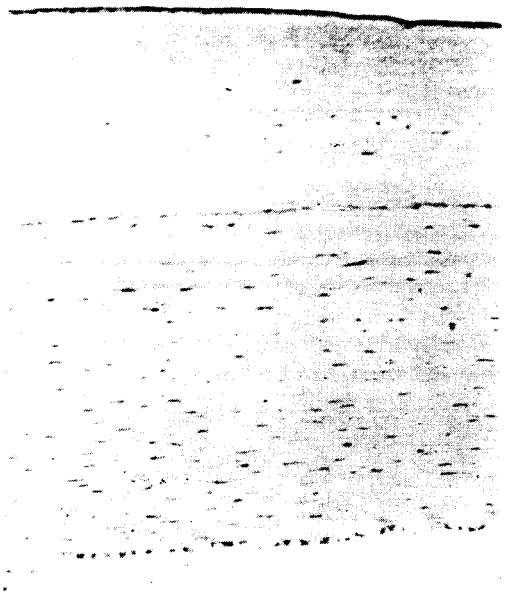

Fig. 3a. *Shows the central graft zone at 26 days post-operatively. Haematoxylin–Eosin; original magnification, × 10.*

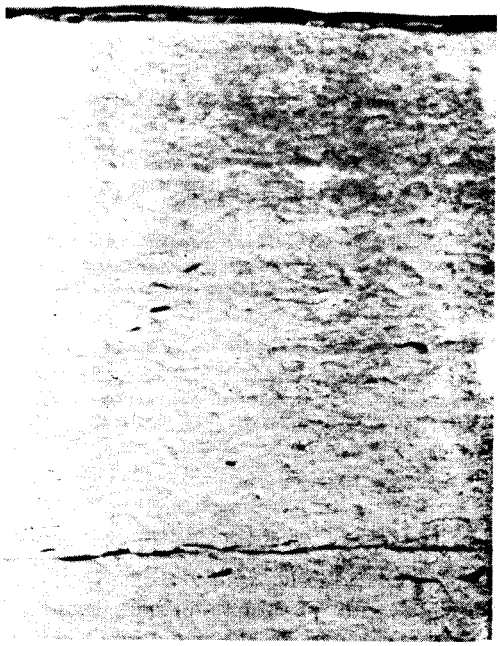

Fig. 3b. *Shows the same area as Figure 3a. Haematoxylin–Eosin; original magnification, × 25.*

nent because of the closeness of fit between the smooth surfaces of the host cornea and the lathed back surface of the lenticule.

Figure 2 of the same eye shows a lower power view of the wing–zone. Larger accumulations of fibrin can be seen filling the space anterior to the graft wing, but there is less fibrin posteriorly. The epithelium of the overlying host cornea has not been stripped from this area which lies peripheral to the trephine mark.

Figure 3a shows the central zone of another graft 26 days postoperatively. The graft has become covered with an attenuated epithelium, the original dead keratocytes have been removed and there is scanty keratocyte repopulation. The fibrin at the graft–host interface has all been cleared and the junction contains a few scattered keratocytes only.

Figure 3b shows the same area as Figure 3a under higher power. The epithelium is seen to be bi-laminar. There are scanty keratocytes in the graft stroma.

Discussion

In vitro studies of our fibrinogen adhesive agreed with the findings of Gestring and Lerner [35] who reported a bond strength better than 100gm/cm^2 for their autologous fibrinogen adhesive, and better than 200gm/cm^2 for the commercial product. The probable reason for the greater bond strength is the difference in the concentration of fibrinogen. The concentration in normal plasma (2–4gm/l) can be raised to around 29gm/l by cryoprecipitation.[33] In Tisseel the concentration is increased by lyophilisation up to as much as 110gm/l. However, during lyophilisation up to 8.5% fibrinogen may be lost, and the addition of synthetic amino acids may be required to maintain solubility and fibrinogen activity.[36,37] It was therefore decided that the added complexity of lyophilisation would not be offset by possible gain in bond strength. Despite the difference in bond strength, there was no significant difference between the results of experimental epikeratophakia performed with Tisseel (19) or with our single–donor fibrinogen.

Frangieh *et al*[38] observed that a temporary, central tarsorrhaphy was beneficial in the postoperative management of the epithelium in epikeratophakia and in this series all retained grafts were fully re–epithelialised by the time the tarsorrhaphy was opened. In this and our earlier series[19] we found that no lenticules were lost once re–epithelialisation had occurred. We previously noted that the topical application of aprotinin aimed at preventing early fibrinolysis of the adhesive, appeared to slow epithelialisation. Unlike the commercially available fibrinogen concentrate, aprotinin was not added to our single–donor fibrinogen and its absence did not appear to have any detrimental effect.

Conclusions

This study has shown that fibrinogen adhesive prepared from single human donors can be effective in securing experimental epikeratophakia grafts in rabbits. The *in vitro* bond strength of our adhesive was slightly weaker than the reported strength of Tisseel, but we found that there was no significant difference in the clinical course and histological appearance of epikeratophakia performed with the two adhesives. The preparation of fibrinogen adhesive by cryoprecipitation from a single donor is a simple and inexpensive technique. The use of autologously derived fibrinogen in humans would avoid the risk of transmission of infection and of immune reaction to foreign plasma proteins. On the basis of these findings it would seem appropriate to evaluate, in the human clinical situation, the technique of sutureless epikeratophakia with autologous fibrinogen adhesive.

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